Development of a 1-concentration-4-step dosimeter protocol for methacholine testing

Rolf Merget a,*, Rudolf A. Jörres b, Evelyn Heinze a, Michael G. Haufs a, Dirk Taeger a, Thomas Brüning a

a BGFA - Research Institute of Occupational Medicine, Bürkle-de-la-Camp-Platz 1, German Social Accident Insurance, Ruhr-University, D-44789 Bochum, Germany
b Institute and Outpatient Clinic for Occupational and Environmental Medicine, Ludwig-Maximilians-University Munich, Germany

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Summary
Methacholine testing is an important diagnostic tool for asthma. Newly available dosimeter and software technology allows for simplification of the test. This study aimed to evaluate a single-concentration dosimeter protocol for methacholine testing by comparison with a multi-concentration dosimeter protocol similar to that recommended by the American Thoracic Society (ATS) (standard protocol).

Fifty young subjects with high pretest probability for bronchial hyperresponsiveness underwent two challenges in randomized order within one week. The novel protocol used a Medic-Aid Sidestream nebulizer and a fixed methacholine concentration of 16 mg/mL. Number and duration of nebulizations were matched to the last four doses of the standard protocol, and results were expressed cumulatively.

The rank correlation between log slopes (n = 50) was 0.86; that between log provocative doses (n = 18), which differed at low values, was 0.58. When requiring a 20% fall in FEV1 at any methacholine dose, 18 subjects were hyperresponsive and 28 normoresponsive in both tests (46/50 concordant). One subject was positive only with the standard, and 3 only with the novel protocol (Cohen's kappa 83%).

The novel protocol for methacholine testing yielded qualitative results similar to those of the ATS multi-concentration protocol, although there were quantitative differences at low doses. However, its design and handling may offer advantages for clinical practice.

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* Corresponding author. Tel.: +49 (0) 23 43 02 45 46; fax: +49 (0) 23 43 02 45 42.
E-mail address: merget@bgfa.de (R. Merget).
Introduction

The assessment of bronchial hyperresponsiveness (BHR) is in widespread use for clinical, clinical-experimental and epidemiological purposes. Based on available methacholine (MCH) challenge protocols, the American Thoracic Society (ATS) proposed a specific dosimeter method involving the administration of five increasing concentrations.1 Despite all efforts for standardization, there are, however, still various methods in use. Among the reasons for this seems to be the intention to simplify the procedure, or the fact that some nebulizers, in particular the DeVilbiss 646 included in the ATS protocol, show weaknesses1 that require appropriate measures.2

One of the points of concern in clinical practice is the use of multiple concentrations as required by most challenge protocols. The preparation of various concentrations needs equipment and is a potential source of error, variability and safety concerns. Conversely, the development of a single-concentration protocol is handicapped by the prohibitive increase in the number of inhalations required to cover the standard ranges of MCH doses. However, current technology allows for additional modification of the nebulization time without changing the basic output rate, and implementation into software renders the use of complex nebulization schemes easy.

It was the aim of this study to develop a single-concentration protocol that covers the required dose range and as closely as possible matches the doses administered in the ATS multi-concentration method. We compared the results of both procedures with regard to the dichotomous outcome of the presence versus absence of BHR, as well as quantitative measures of response, in a population of subjects with a high pretest probability of BHR.

Material and methods

Study subjects

Fifty-six subjects were recruited from medical students. All answered at least one question of the ATS questionnaire1 affirmatively (physician-diagnosed asthma ever; hospitalized for asthma ever; (chronic or recurrent) respiratory disease as a child; asthma symptoms within the last two weeks). Three subjects did not perform the second challenge, and three were excluded due to unacceptable spirometry. Thus, the final population comprised 50 subjects (22 females; median age 25, range 22–24 years; median FEV1 101.9, range 73.9–145.7% predicted (baseline of standard protocol) and 103.9, range 81.1–139.3% predicted (baseline values of novel protocol)). All subjects denied to have experienced a lower respiratory tract infection within 6 weeks prior to measurements. None of them showed one of the accepted contraindications of MCH challenge or a medication that is considered to influence the results. The study was approved by the Ethics Committee of the Ruhr-University and all subjects gave their informed consent.

Study design

Subjects participated in the two challenges in randomized order within one week. Tests were done between 8 a.m. and 3 p.m., paying attention to performing both challenges in one individual at comparable times of the day (median time interval 65, interquartile range 20–146 min).

Methods

Standard protocol according to ATS

The protocol used for comparison was based on the dosimeter protocol recommended by the ATS.1 This involves five concentrations administered in five steps by a DeVilbiss 646 nebulizer (DeVilbiss, Malsch, Germany), without initial inhalation of the diluent (Table 1). Each concentration was given in five consecutive slow inspirations from functional residual to near total lung capacity, while the nebulizer was actuated over 0.6 s. Inspiratory airflow was kept close to 1 L/s by observation of a visual scale. The time interval between consecutive steps was 5 min, with a range from 4 to 6 min in single subjects. Spirometry was performed at baseline as well as 90 s after inhalation of each concentration. At baseline and after the last step spirometry comprised three acceptable manoeuvres, whereas only one acceptable manoeuvre was required after the other steps.

Compared to the ATS protocol,1 three minor modifications were incorporated. Firstly, the nebulizer was actuated 0.5 s after the start of inspiration to ensure a significant airflow upon nebulization. Secondly, we omitted breathholds after inhalation to facilitate comparability, as these were considered unfeasible in the novel protocol comprising a greater number of consecutive inhalations. Thirdly, spirometry was performed at only one time point, as prior to spirometry bodyplethysmography was done (data not shown). This protocol is called standard protocol throughout the manuscript. The end-of-test criterion was either a ≥ 20% fall in FEV1 compared to baseline or having reached the highest concentration of MCH.

MCH (Synopharm, Barsbüttel, Germany) was diluted with isotonic saline. Nebulizations were performed by an APSpro dosimeter (Viasys Healthcare, Höchberg, Germany) and the nebulizer filled with 2 mL solution. The straw of the nebulizer had been fixed2 to minimize the previously reported variability of output3; the output was 9 μL per actuation.

Novel single-concentration protocol

Based on the nominal output of the DeVilbiss 646 nebulizer (900 μL/min), the number of 5 inhalations of 0.6 s duration and the increasing concentrations of the standard protocol, the dose delivered at each step was computed (Table 1). We merged the lowest two concentrations into a single equivalent concentration. Using the nominal output of the Medic-Aid Sidestream nebulizer (240 μL/min; Viasys Healthcare, Höchberg, Germany) that was utilized in this protocol and the fixed MCH concentration of 16 mg/mL, a scheme of nebulization times and numbers was developed (Table 2) that comprised the same dose sequence as the standard protocol (Table 1). The Medic-Aid nebulizer was
combined with the electronic, software-controlled APSpro dosimeter (Viasys Healthcare) that ensured a reliable switching of the number and duration of nebulizations within the procedure.

As provocative substance we used MCH solved in phosphate-buffered saline (Provokit®/C226; Lindopharm, Hilden, Germany). As in the standard procedure, nebulization was initiated 0.5 s after start of each of the consecutive slow inspirations to near total lung capacity, without breath-hold, and spirometry was performed 90 s after inhalation.

Quality control
All tests were performed under comparable conditions in a climate room at 24 °C and a relative humidity of 50%. Nebulizer outputs were assessed weekly by weighing. There were no trends towards increasing or decreasing outputs over time, and in nearly all measurements the output was within ±10% of the nominal value of the nebulizers, as recommended. All spirometric measurements were examined by trained technicians and had to fit acceptability criteria. Overall, 84% of repeated baseline measurements, and 81% of repeated measurements performed at the last dose administered, met both the FEV1 and FVC reproducibility criteria.

Analysis
For each challenge, PD20 FEV1 was derived by linear interpolation, whereby doses were plotted logarithmically and responses linearly. In tests with a positive response at the first step, a virtual initial dose of 0.1 µg MCH was assumed to calculate a provocative dose. As an alternative measure which allowed to quantify also challenges in which a 20% fall in FEV1 was not achieved, the slope of the dose–response curve was calculated.

Data analyses were performed using log-transformed (base 10) slopes for all subjects and log-transformed (base 10) PD20 FEV1 for those showing a ≥ 20% fall in FEV1 in both tests. Correspondingly, geometric mean values and standard deviations (SD) were computed, geometric SD being expressed as dimensionless variability factor. To quantify the agreement between values, Spearman’s rank correlation coefficients (r_s) were calculated, and log values were compared by the t-test for paired samples. Additionally, standard linear regression analysis was performed. In scatter plots orthogonal regression lines are depicted, and approximate confidence intervals are given in the respective figure legends. We also performed a Bland & Altman analysis to elucidate the relationship between the values by the two protocols. The agreement of the binary classification according to different cut-off doses of BHR was tested by Cohen’s kappa. Statistical significance was assumed if p < 0.05, and 95%-confidence intervals (95%-CI) were given where appropriate. All analyses were performed using the statistical package SAS 9.1 (Cary, NC, USA).

Results
All subjects tolerated both challenge protocols well. One subject showed a 29% fall in FEV1 after inhalation of the initial dose of 15 mg MCH of the novel protocol, and a 32% decrease after inhalation of 3 mg MCH of the standard protocol. A second subject showed a 27% fall in FEV1 after the second step of the standard protocol. All other ≥ 20% FEV1 decreases occurred at doses >15 µg MCH with both protocols. The mean (SD) maximal fall in FEV1 at the end of the test was 15.8 (10.0)% from baseline with the standard protocol and 15.5 (9.3)% with the novel protocol.

<table>
<thead>
<tr>
<th>Step no</th>
<th>Nebulization time per breath (s)</th>
<th>No of breaths (n)</th>
<th>MCH concentration (mg/mL)</th>
<th>MCH dose (µg)</th>
<th>Cumulative MCH dose (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.234</td>
<td>1</td>
<td>16</td>
<td>15</td>
<td>15</td>
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<tr>
<td>2</td>
<td>0.352</td>
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<td>16</td>
<td>45</td>
<td>60</td>
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<tr>
<td>3</td>
<td>0.563</td>
<td>5</td>
<td>16</td>
<td>180</td>
<td>240</td>
</tr>
<tr>
<td>4</td>
<td>0.865</td>
<td>13</td>
<td>16</td>
<td>720</td>
<td>960</td>
</tr>
</tbody>
</table>

Table 2 Novel single-concentration-4-step dosimeter protocol. The output of the Medic-Aid nebulizer used in the calculations was 240 µL/min.
Geometric mean (geometric SD) of PD_{20}FEV_{1} was 78 (6.8) µg for the standard protocol and 113 (3.7) µg for the novel protocol; these values were not significantly different (p = 0.21). Mean PD_{20}FEV_{1} was greater by 0.53 (95%-CI −0.33 to 1.4) doubling doses in the novel compared to the standard protocol. Geometric mean (SD) of log slopes was 22.9 (6.2) and 21.7 (5.5)%/mg which was not significantly different from each other (p = 0.68).

Log PD_{20}FEV_{1} of both protocols correlated with each other (n = 18, rs = 0.58, p = 0.0122; Fig. 1). Regression analysis (novel versus standard protocol) yielded a slope of 0.53 (95%-CI 0.30–0.76) and an intercept of −0.36 (−0.67 to −0.04). If PD_{20}FEV_{1} values ≤ 15 µg (n = 2) were omitted, the slope was 0.42 (95%-CI 0.11–0.74) and the intercept −0.45 (−0.79 to −0.10). The Bland & Altman plot (Fig. 2) indicated that the difference between PD_{20}FEV_{1} values depended on their magnitude, in agreement with the fact that the slope was significantly different from one.

Log slopes showed a mean difference between novel and standard protocol of −0.05 (95%-CI of −0.03 to 0.21). There was a correlation between log slope (rs = 0.86, p < 0.0001; Fig. 3), whereby the linear regression line showed a slope of 0.80 (95%-CI 0.67–0.94) and an intercept of 0.25 (0.04–0.46). The respective Bland & Altman plot (Fig. 4) indicated that the difference between slopes did not depend on their magnitude.

If BHR was defined by a ≥ 20% fall in FEV_{1} after any dose of MCH, 18 subjects were rated as positive and 28 as negative in both tests (46/50 concordant). One subject was positive only with the standard protocol and three only with the novel protocol (Cohen’s kappa of 83% (95%-CI 68–99%).

If a ≥ 20% fall in FEV_{1} after cumulative doses of ≤240 mg was required, 11 subjects were rated as positive in both tests and 36 as negative (47/50 concordant). Three subjects were positive only with the novel protocol (Cohen’s kappa of 84% (95%-CI 67–100%).

**Discussion**

The present study demonstrated a reasonable agreement between a novel single-concentration dosimeter protocol for MCH testing and a standard multi-concentration protocol. This was true for dose response slopes as well as...
for the recognition of BHR by different cut-off values of provocative doses. At low values, however, provocative doses differed between both methods.

To evaluate the novel method, we referred to a newly designed multi-concentration dosimeter protocol recommended by the ATS, similar to those used previously. As multiple concentrations involve potentially time-consuming and error-prone dilution steps, we aimed at developing a single-concentration protocol the doses of which closely matches those of the ATS protocol. This protocol should be as simple as possible in its practical use, by delegating the major technical issues involved to the software control of the nebulizer.

The nebulization scheme required for this was derived from a comparison of nebulizer outputs. To our knowledge, data on the aerosol output of the DeVilbiss 646 for short actuation are not available. In the Medic-Aid nebulizer, the output at short actuation corresponded to 64–85 \( \mu \)L/min, about half the reported steady-state value (160 \( \mu \)L/min). Using nebulization durations of 0.6 s and 10 actuations we recorded a weight loss of 240 \( \mu \)L/min ± 10% when using the pressure-stabilized APSpro dosimeter at a pressure of 1.3 × 10^5 Pa and a flow of 9.6 L/min. Among the reasons for the differences in output might be a lower driving pressure or the lower filling volume of 1 mL used previously. Determination of aerosol output by weight might be affected by condensate, if subjects exhale through the nebulizer (see 12). As this is unlikely to affect the actual output, the variability of 10% was probably an upper bound for the true variability. Clearly, determinations of aerosol output cannot substitute for a comparative study, as biological and technical doses can differ and results are not predictable solely from technical data.

While we used MCH chloride and isotonic saline in the standard method, the single-concentration protocol involved a commercially available solution of MCH chloride in phosphate-buffered saline. Both solutions were matched with regard to MCH content. Saline was chosen to achieve comparability with ATS recommendations. In contrast, the substance approved by German authorities for routine inhalation challenges is that solved in phosphate-buffered saline. We considered it unwise not to use this in the novel protocol especially designed for clinical practice, as well as for pharmacological trials. There seem to be no published data on a comparison of MCH formulations, but effects of diluents should have been related to ionic composition and/or pH, which, however, are not likely to play a role at the low amounts of fluid delivered. It is therefore unlikely that the use of different MCH sources, while matching the amount of active substance, has considerably influenced our results, especially the differences between provocative doses.

In both protocols the amount of MCH was expressed as cumulative dose. By the nature of its design the novel protocol favours a cumulative evaluation, whereas the ATS preferred provocative concentrations to facilitate calculations. Even more, the novel protocol required physiological cumulative concentration of inhalations within the short time interval (≤1 min) of one dose step. The fact that there was a dose-response allowing to compute PD\(_{20}\)FEV\(_1\) values showed this assumption to be satisfied. With regard to data evaluation, non-cumulative concentrations and cumulative doses are linearly related to each other, and the choice between them amounts to a rescaling of results. Thus the relationships illustrated in Figs. 1 and 3 are essentially unaffected by choosing cumulative versus non-cumulative evaluation.

An issue different from that regarding the expression of results is the physiological cumulative of MCH effects which depends on the time between subsequent inhalations. We kept this time interval as close as possible to 5 min in both protocols. The sources of minor variation (±1 min) encountered in single patients were of technical nature and are unlikely to have influenced the results. For example, assuming a 20% cumulation of doses at 5-min intervals and an exponential decay of this effect, a variation of ±1 min would lead to a change in effective dose of less than 10%. The regression line between the values of PD\(_{20}\)FEV\(_1\), significantly differed from the line of identity, however in most patients the difference between these values at least ranged within two doubling doses of MCH relative to the regression line (Fig. 1). This was only slightly higher than the range of ±1.5 doubling concentrations considered acceptable in repeated challenges using the same protocol.

Log slopes which could be computed in all subjects were close to the line of identity even at high degrees of BHR, and the Bland & Altman plot did not indicate a systematic difference between the two methods. In contrast, PD\(_{20}\)FEV\(_1\) showed a difference despite the equivalence of nebulizer outputs. This, however, did not seem to affect the recognition of BHR, which we consider as a major result. The fact that the relationship of log slopes to each other and that of PD\(_{20}\)FEV\(_1\) values at low but not at high doses differed, suggests a difference in the shape of dose–response curves. To explain the differences, one might try to invoke potential differential effects of MCH concentration and cumulation. At all steps except the last one, MCH concentration was higher in the novel compared to the standard protocol. If concentration within aerosol particles, in addition to total dose, was of importance, there should have been stronger responses, i.e. lower PD\(_{20}\)FEV\(_1\), in the first steps of the novel protocol, in contrast to the...
obstructive airway responses to diluent are rare. One
rable, both of them being based on five nebulizations of
and the fourth step of the standard protocol were compa-
standard protocol by merging the first and second step into
When developing the novel protocol we shortened the
study14 which compared a single-concentration-seven-step
function responses were similar. We thus believe that the
protocol. In the course of both challenges, lung
(32% from baseline) at the lowest dose of the standard
presented here are indispensable for the evaluation of
output, and that empirical comparisons such as the one
vehicle. It seems in accordance with this assumption
PD20FEV1 was comparable at high doses and different at
nebulizers or different modes of action of the same nebu-
devices. Thus the differences between PD20FEV1
numbers of inhalations (one or two versus five) would also
not explain that PD20FEV1 values of the novel protocol were
were elevated at low doses. There remains as the most likely
short actuation times of the Medic-Aid device resulted in a lower deposited aerosol fraction rela-
to weight. It seems in accordance with this assumption
PD20FEV1, corresponding to the third step of the novel
and the fourth step of the standard protocol were compara-
both of them being based on five nebulizations of
0.5 s duration. Thus the differences between PD20FEV1
values appear to emphasize the argument that different
nebulizers or different modes of action of the same nebu-
zation. Thus, the assumption of different bronchodilator responses at low
numbers of inhalations (one or two versus five) would also
not explain that PD20FEV1 values of the novel protocol were
elevated at low doses. There remains as the most likely
possibility that short actuation times of the Medic-Aid
device resulted in a lower deposited aerosol fraction rela-
tive to weight. It seems in accordance with this assumption
that PD20FEV1 corresponding to the third step of the novel
and the fourth step of the standard protocol were compara-
ble, both of them being based on five nebulizations of
at low doses of the standard protocol, and about the same response after the first dose
of the novel protocol. In the course of both challenges, lung
function responses were similar. We thus believe that the
novel protocol offers a similar margin of safety as the
standard protocol. Our data extend those of a previous
study13, which compared a single-concentration-seven-step
protocol using 32 mg/mL MCH and the Medic-Aid nebulizer
with a multi-concentration protocol based on the DeVilbiss
646.11 Our method was designed to be shorter and simpler
than this. Due to its easier handling, the single-concentra-
tion protocol was favoured by the technicians compared to
the standard protocol.

Both protocols did not comprise inhalation of the diluent
prior to the first MCH dose. Although often incorporated,
diluent inhalation is not considered mandatory,1 and
obstructive airway responses to diluent are rare. One
reason for inclusion might be to familiarize subjects with the
procedure. However, the participants studied were
cooperative in performing the required manoeuvres. When
implementing the protocol in clinical practice, measuring
untrained patients, an initial diluent inhalation could be
easily added.

In conclusion, the results of our study suggest that - at
least for qualitative purposes such as deciding on the
presence of BHR - a simplification of MCH inhalation chal-
genades can be achieved by using a single concentration at
varying nebulization times and numbers. The novel protocol
avoided the preparation of different dilutions, which might
be not very feasible for clinical routine in outpatient
clinics. The simplification by using a single concentration
could be achieved by software-controlled nebulization
without introducing additional sources of error. Although
there was a high concordance of both tests with respect to
classification of BHR, there was an unexplained systematic
deviation at low doses that could, however, be described by
a linear regression line. Further studies, possibly using
further improved nebulizers, might achieve complete
quantitative comparability also at low doses.

Conflict of interest

There is no conflict of interest for any author.

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